The effect of inhalation volume and breath-hold duration on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking

Alan K Armitage[1], Michael Dixon[2], Barrie E Frost[3], Derek C Mariner[3] and Neil M Sinclair[3]*

1. Sycamore Lodge, Abbey Road, Knaresborough, North Yorkshire, HG5 8X, UK

2. British American Tobacco, Globe House, 4 Temple Place, London WC2R 2PG, UK

3. Rothmans International, Group Science and Technology Centre, Tilbrook, Milton Keynes, UK

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Correspondence to:

Dr DC Mariner, British American Tobacco, R & D Centre, Regents Park Road,

Southampton. SO15 8TL. United Kingdom

Fax: +44 (0) 23 8079 3076

Tel: +44 (0) 23 8079 3025

Email: derek_mariner@bat.com

Abstract. Objective. To investigate the influence of inhalation depth and breath-hold duration on the retention of nicotine and solanesol in the human respiratory tract and on nicotine uptake into venous blood for a 10mg "tar" yield cigarette typical of the US market. Subjects/materials/method. Ten current, inhaling smokers of fifteen to twenty 8-12mg 'tar' yield cigarettes/day who were acclimatised to the test cigarette. When smoking this cigarette in the study, subjects were requested to puff normally but at one puff per minute and for seven puffs per cigarette. The subjects' puff volumes were recorded and they were required to prevent any smoke escaping from the mouth between puffing and exhalation. In a first series of experiments, a fixed volume of air (0, 75, 250, 500 or 1000mL) was inhaled after each puff in order to give a controlled 'depth' of inhalation. The inhalation was drawn from a bag containing the required volume of air. Following a 2s breath-hold, subjects exhaled normally, with the first exhalation after each puff passing through a single acidified filter pad for collection of the non-retained nicotine and solanesol. Blood samples were taken before and at intervals during and after smoking for the sessions with 0, 75 and 500mL inhalation volumes. Another series of experiments was conducted with a fixed inhalation volume (500mL) and two further breath-hold durations (0 and 10s) in addition to 2s from above. Nicotine and solanesol retentions were measured for each breath-hold duration. Results. The amounts of nicotine retained within the respiratory system, expressed as a percentage of the amount taken into the mouth, were consistently higher than the corresponding values for solanesol in all five inhalation conditions (0 - 1000mL, 2s breath-hold). Nicotine retention increased from 46.5% at zero inhalation to 99.5% at 1000mL inhalation (2s breath-hold) and from 98.0% at zero breath-hold to 99.9% at 10s breath-hold (500mL inhalation). Solanesol retention increased from 34.2% at zero inhalation volume to 71.9% at 1000mL inhalation and from 51.8% at zero breath-hold to 87.6% at 10s breath-hold (500mL inhalation). Plasma nicotine decreased from pre-smoking levels after zero inhalation. After 75mL inhalation, plasma nicotine levels were significantly greater than for zero inhalation but not significantly less than after 500mL inhalation except at the time of maximum nicotine concentration. Conclusion. In every experimental condition, a higher percentage of nicotine than solanesol was retained

within the respiratory tract. The difference in retention of the moderately volatile nicotine and the involatile solanesol is consistent with the efficient retention in the airways of gaseous nicotine that has evaporated from smoke particles. With no inhalation (the smoke was held in the mouth for two seconds), approximately 50% of the nicotine was retained but there was no increase in venous plasma nicotine levels. With 75mL inhalation, 90% of the nicotine was retained, and with a 500mL inhalation, virtually all of the nicotine was retained. Although solanesol retention increased with depth of inhalation and with breath-hold duration, nicotine retention was almost complete even at shallow inhalations and short breath-hold durations.

Key words: nicotine - solanesol - retention - inhalation - cigarette smoking

Introduction

Nicotine is predominantly present in the particulate phase of mainstream cigarette smoke as it exits the cigarette (Houseman *et al.* 1973, Lewis *et al.* 1995, Stevens and Borgerding 1999). Mainstream cigarette smoke consists of particles with mass mean aerodynamic diameters <0.5μm (Chen *et al.* 1991) which, theoretically, would be expected to penetrate the small airways and alveolar region of the lungs when inhaled. It has generally been assumed that nicotine is absorbed from these regions of the lungs during smoke inhalation and it has been suggested that this results in a rapid transport (<10s) of a bolus of nicotine from the lung to the brain following the inhalation of each puff (Russell and Feyerband 1978).

Recent publications have questioned these assumptions regarding the mechanisms of smoke deposition and nicotine absorption from the lung. Frost *et al.* (1998) measured the amounts of nicotine retained within the respiratory tracts of four smokers and observed ca. 90% nicotine retention following even a very shallow inhalation (<100mL). The authors concluded from the different retentions of nicotine and 'tar' at various depths of inhalation that nicotine evaporates from the smoke particle during inhalation and is absorbed in the upper airways as a vapour.

Rose *et al.* (1999) reported that the delivery of nicotine into arterial blood following cigarette smoke inhalation was substantially less and slower than would have been predicted if nicotine was absorbed predominantly from the alveolar region of the lung. The authors postulated that uptake of nicotine into the respiratory tract tissue considerably slows the entry of nicotine into the systemic circulation.

The current study was designed to determine the contribution of particulate deposition and nicotine evaporation to the respiratory retention of nicotine by examining the influence of changing inhalation patterns on the retention of nicotine and a non-volatile particulate phase marker, solanesol, in the mouth

and respiratory tract. Additionally, the study attempted to determine the influence of the inhalation patterns and sites of nicotine deposition on the uptake of nicotine into the systemic circulation.

Subjects, materials and methods

Subjects

Ten male smokers, who had given informed consent, participated in the study. All were aged between 21 and 40 years and regular inhaling smokers of fifteen to twenty 8-12mg "tar" yield cigarettes per day. Regular smoking status was confirmed by saliva cotinine measurements exceeding 100ng/mL (range 223 - 564ng/mL) in accordance with Stirling *et al.* (1996). The subjects were selected to be within normal limits for body mass index and respiratory function. Test cigarettes were provided to the subjects two days prior to experimental sessions to allow acclimatisation to the tobacco blend style, which was different from their normal cigarettes. Subjects were asked to smoke only the test cigarette during this period and to keep a record of their smoking activity. The use of nicotine gum and/or nicotine patches was prohibited. Subjects were asked to refrain from smoking for a minimum of one hour before each test smoking session. When blood sampling was to be performed, cessation for at least four hours was required.

Cigarettes

The test cigarette used in this study was a 10mg "tar" product with a tobacco blend typical of the US market. The mainstream smoke yields for machine smoking of this cigarette under ISO conditions (one puff/min, puff duration: 2s, puff volume: 35mL, butt length: overwrap+3mm) were as follows: "tar" 9.6mg; nicotine 0.67mg; CO 10.1mg (ISO 4387:1991; ISO 10315:1991; ISO 8454:1995). The cigarettes were selected for weight and pressure drop (mean±5%) to reduce variability.

Experimental procedure

For each experimental smoking session, the test cigarette was smoked through a cigarette holder attached to a smoking analyser which recorded the puff volumes, puff durations and puff times. This system was based on the method originally described by Creighton *et al.* (1978). These records were later used to reproduce the subjects' puff volumes and times on a smoking machine in order to determine

the amounts of nicotine and solanesol in the smoke generated by each subject on each occasion. After smoking, the cigarette was extinguished in solid CO_2 and the butt length measured.

Subjects were asked to take seven puffs, at 60s intervals, and to prevent any smoke escaping from the mouth after puffing and before exhalation (i.e. no waste smoke). As required by the protocol for a given session, a fixed volume of air was inhaled after each puff. The inhalation was taken from a collapsible anaesthetic bag containing the specified volume of air (Figure 1A) and the breath was held for the specified period.

Insert Figure 1 here

The experimenter timed the breath-hold duration and instructed the subject when to exhale. The first exhalation after each puff was directed through an acidified 92mm Cambridge filter pad (Figure 1B) and was vacuum assisted to overcome the effect of filter resistance; this facilitated a more natural exhalation pattern. All such exhalations from one cigarette were directed through one filter pad. The acidified Cambridge filter pads were prepared in a smoke-free environment by soaking in 1N H_2SO_4 for approximately 15 minutes. They were air- dried at room temperature for approximately 24 hours and stored in an airtight container.

Inhalation and mouth/breath-hold manoeuvres

Reproducibility. To assess reproducibility of the sessions, two subjects inhaled 500mL post-puff for five smoking sessions and two subjects inhaled 75mL post-puff for five smoking sessions, all with a 2s breath-hold.

Inhalation depth. Volumes of 75, 250, 500 and 1000mL were used in the collapsible bag to control the volume of the post-puff inhalation. On each occasion, inhalation was followed by a two second breath-

hold. Additionally a zero inhalation (mouth hold) condition was included by instructing the subjects to puff and hold the smoke in their mouths for 2 seconds prior to exhaling.

The range of inhalation volumes used in this study was chosen to bracket the 'normal' inhalation volume range of 413-913mL, (US DHHS 1988). The 75mL inhalation volume was intended to be a 'dead-space' inhalation i.e. to allow the smoke to penetrate as far as the conducting airways but not into the gas exchange or alveolar region. Previous work on the inhalation of carbon monoxide (Guyatt et al. 1981) suggested that alveolar absorption does not occur with inhalation volumes less than 120mL in adult males.

Breath-hold duration. In addition to the 2s breath-hold at the range of inhalation volumes, 0 and 10s breath-hold manoeuvres were performed after a 500mL inhalation.

Determination of nicotine and solanesol delivered to human volunteers.

The puff volumes, durations and intervals recorded during the smoking of each cigarette were reproduced using a custom built smoking duplicator at Rothmans International Group Science and Technology Centre, Milton Keynes, UK. However, a sine wave puff profile was used instead of the exact profile generated by each subject. Creighton and Lewis (1978) reported that there were negligible differences in nicotine delivery between puff shapes for a given puff volume and duration.

The nicotine and solanesol deliveries from the duplicator smoking sessions were determined using the instrumental procedures in ISO Method 10315:1991 and ASTM Method D6271-98, respectively.

Determination of exhaled nicotine and solanesol

After use, the acidified Cambridge filter pads were removed from the holder and were extracted by shaking in alkali and di-isopropyl ether containing quinoline as internal standard. The aqueous layer was separated from the organic layer and extracted twice more with internal standard solution. The combined extracts were stored at -20°C awaiting analysis. Nicotine was analysed by gas chromatography (based on ASTM Method D5075-96e1). After evaporation of the extract to dryness and dissolution in methanol, solanesol was measured by high performance liquid chromatography (based on ASTM Method D6271-98).

Calculation of nicotine and solanesol retention

The respiratory retentions of nicotine and solanesol were calculated as follows:

Retention (%) = 100 * (amount delivered – amount exhaled) / amount delivered

Blood sampling

Blood samples were taken from each subject during the zero, 75mL and 500mL inhalation, 2s breath-hold conditions, and from two subjects during the reproducibility trial.

A cannula was inserted into an ante-cubital vein and 5mL blood samples were taken at the following times:

1 min before the lighting puff (puff 1)

3.5 min after lighting (30s after puff 4)

7 min after lighting (1 min after last puff)

10, 15, 20, 40 and 60 min after lighting.

Blood samples were collected into heparinised tubes and centrifuged. Plasma was transferred to polypropylene tubes and stored at -20°C to await nicotine analysis, which was completed within three months.

Additional 5mL blood samples were taken pre-smoking and at 10 minutes after lighting. These were collected into EDTA anticoagulated tubes for carboxyhaemoglobin (COHb) determination.

Determination of plasma nicotine

Nicotine was extracted from plasma samples by mixing with 35% ammonia solution, dichloromethane and quinoline internal standard solution and analysed by gas chromatography (based on ASTM Method D5075-96e1).

Where plasma samples contained nicotine levels between 2ng/mL, the limit of detection (LoD), and 5ng/mL, the limit of quantification (LoQ), a value of 2.5ng/mL (half LoQ) was assumed. This approach has been used previously, eg Nehls and Ackland (1973).

Determination of COHb in whole blood.

All assays were performed within one hour of sample collection using a blood gas analyser (Instrumentation Laboratory Synthesis 35). Carboxyhaemoglobin boost was determined by subtracting the pre-smoking value from the post-smoking value.

Statistical methods

Statistical evaluation was by Analysis of Variance (ANOVA) and by Fisher's test for the comparison of means (individual error rate 0.05) using Minitab Version 13.1 (Minitab Inc, State College, Pennsylvania, USA). No outlier testing was undertaken.

Pharmacokinetics

Pharmacokinetic parameters were derived from the plasma nicotine data using WinNonlin Version 1.5 (Pharsight Corporation, Mountain View, California, USA).

Results

Protocol compliance

The protocol required familiarisation sessions and 90 study sessions. It was conducted over a five-week period. The following deviations from protocol occurred:

- two subjects could not be cannulated on one occasion each, thus the 75mL inhalation plasma data set is based on 8 rather than 10 subjects
- the smoking behaviour records for two sessions could not be duplicated because the records were corrupted.

Reproducibility

The reproducibility data are shown in Appendix 1.

Table/text of puff vols, N & S deliveries

Effect of inhalation volume on nicotine and solanesol retention within the respiratory tract.

Five inhalation volumes with a 2s breath-hold were studied for the test cigarette (Figure 2A).

Insert Figure 2 here

The retentions of nicotine, expressed as a percentage of the delivered amount, were consistently higher than the corresponding values for solanesol in all conditions. Nicotine retention with zero inhalation (46.5 ±8.6%) was lower than at all other inhalation volumes ($p<0.05$). Nicotine retention at 75mL inhalation (89.8 ±11.4%) was less than at higher volumes ($p<0.05$). There were no statistically significant differences between nicotine retentions at 250, 500 and 1000mL inhalation volumes (96.4 ±1.5%, 99.0 ±0.5% and 99.5 ±0.3%, respectively). Solanesol retentions ranged from 34.1% with no inhalation to 70.9% with an inhalation volume of 1000mL. The solanesol retentions at 75 and 250

inhalation volumes did not differ significantly, nor did those at 500 and 1000mL inhalation volumes. All other differences were statistically significant ($p<0.05$).

Effect of breath-hold duration on nicotine and solanesol retentions within the respiratory tract

The effect of breath-hold duration (0, 2 and 10s) was studied at an inhalation volume of 500mL (Figure 2B). With a 0s breath-hold, 98.0 ±0.8% of the nicotine delivered was retained. Nicotine retention increased to 99.9 ±0.1% with a 10s breath-hold. Although the arithmetic differences between nicotine retention at the different inhalation volumes were small they were statistically significant ($p<0.05$).

Solanesol retention increased significantly ($p<0.05$) as breath-hold duration increased from 0 sec (51.8 ±5.9%) to 2 sec (68.0 ±7.5%) to 10 sec (87.6 ±2.4%).

Effect of inhalation volume on plasma nicotine levels

Pre-smoking plasma nicotine levels were not significantly different for the three inhalation volumes (Figure 3).

Insert Figure 3 here

Plasma nicotine levels did not change significantly from the pre-smoking values following the mouth-hold (zero inhalation) manoeuvre. Following the 75mL inhalation, plasma nicotine levels were significantly higher ($p<0.05$) than after the mouth-hold except at 40 and 60 minutes. Plasma nicotine levels at every time point after the 500mL inhalation were significantly higher ($p<0.05$) than those following the mouth-hold manoeuvre. Plasma nicotine levels following the 500mL inhalation were consistently higher than those following the 75mL inhalation, however, the difference was statistically significant ($p<0.05$) only at the 7 minute sample point. This was the point of maximum plasma nicotine concentration

Effect of inhalation volume on COHb levels

Carboxyhaemoglobin levels increased significantly after the 75 and 500mL inhalations (boosts: 0.40% ±0.28% and 0.83% ±0.30%, respectively; $p<0.05$) but there was no significant change after the mouth-hold (zero inhalation) (boost: -0.03% ±0.31%; NS).

Smoking Behaviour

The experimental procedure for this study controlled all aspects of smoking behaviour except puff volume and, of course, nicotine metabolism.

Subjects were very consistent in their individual total puff volumes per cigarette across the seven experimental smoking sessions. For the ten subjects, mean puff volumes across the sessions ranged from 40.9+/- 2.2 ml to 87.4 +/- 16.2ml. The mean puff volume for all subjects and sessions was 59.7 +/- 10.7ml which was within the range of published mean puff volumes (range 21ml to 66ml USDHSS 1988).

There were no statistically significant differences between replicates ($p>0.05$, two-way ANOVA, General Linear Model for unbalanced data) in the reproducibility section but there were differences between subjects. This indicates that the methodology is robust and capable of detecting differences between manoeuvres.

Discussion

The principal findings of this study are: (1) that nicotine retention was greater than solanesol retention in all manoeuvres; (2) that 90% or more of the nicotine is retained even at an inhalation volume as low as 75mL and (3) that 50% retention of nicotine during the mouth-hold manoeuvre did not increase plasma nicotine concentrations.

The first finding has implications for the mechanisms of retention of nicotine and solanesol during smoking. Solanesol does not evaporate from aerosol particles at body temperature, even at high dilution. It can, therefore, be retained in the airways and lungs only by deposition of the smoke particles. This deposition results from inertial impaction, Brownian diffusion and gravitational sedimentation (Dixon *et al.* 2000). The greater retention of nicotine demonstrates that it did not all remain in the aerosol particles with solanesol, otherwise the retentions of the two compounds would be expected to be the same, and supports the hypothesis that nicotine evaporates from the smoke particles, diffuses to and is absorbed into the surface of the airways. This is consistent with the data reported by Frost *et al.* (1998) for a smaller number of subjects using similar methodology, and by the 'in vitro' experiments on the evaporation of nicotine from particles by Lewis *et al.* (1995).

The second finding that 46.5 ±8.6% nicotine retention in the mouth-hold manoeuvre and 89 ±12% in the 75mL inhalation manoeuvre strongly suggests that substantial amounts of nicotine can be retained within the upper respiratory tract and will not reach the alveolar region of the lung. This is consistent with recent observations of nicotine absorption by Rose *et al.* (1999) who claimed that rises in arterial levels of nicotine were more than 10 times lower than would have been expected if the nicotine were absorbed directly from the alveolar region of the lung. Our finding that, even with a modest inhalation volume of 250mL (the 'normal' inhalation volume range is 413-913mL, US DHHS 1988) and 2s breath-hold, 96.4 ±1.5% of nicotine was retained, means that there is little scope for further retention by

increasing inhalation volume or breath-hold time. This observation is consistent with the work of Zacny *et al.* (1987) who found that increasing inhalation volume from 10% to 60% vital capacity, or post inhalation breath-hold time from zero to 16 seconds did not increase nicotine 'boosts' (post minus pre-smoking venous nicotine levels).

In contrast, solanesol retention in the respiratory tract increased significantly with increasing inhalation volume and breath-hold duration.

The third finding relates to nicotine retention and uptake in the mouth. In this study 46.5 ±8.6% nicotine was retained after a 2s mouth-hold. This is consistent with results from Frost *et al.* (1998) who found 32.6 ±15.7% retention after puffing with immediate exhalation. Our observations that venous nicotine levels did not increase following the mouth-hold (zero inhalation) condition confirm results previously reported by Gori *et al.* (1986) and Zacny *et al.* (1987), however neither group measured retention. Our data are consistent with work using inhaled nicotine vapour, where the nicotine is found primarily in the mouth, oesophagus and stomach (Lunell *et al.* 1996, Bergstrom *et al.* 1995). These and related studies (e.g Russell *et al.* (1987), Schuh *et al.* (1997), Molander *et al.* (1996)) indicate that nicotine transfers poorly from the mouth to the systemic circulation. Our finding that there is greater retention of nicotine than solanesol in the mouth suggests that evaporation of nicotine from particles has already started at this point. However, our results indicate the majority of nicotine retention in the mouth results from particulate-bound nicotine deposition.

Particles of the size present in cigarette smoke might be expected to be deposited downstream from the mouth and upper airways in the more distal regions of the lungs (Martonen and Musante 2000). However, Martonen and Musante concluded that cigarette smoke exhibits cloud motion, a phenomenon

which increases deposition in the upper airway region. This may be the explanation for the high levels of solanesol retention within the mouth and upper airways found in our study.

Guyatt (1981) suggested that alveolar absorption of carbon monoxide does not occur with inhalation volumes less than 120mL in adult males. It was, therefore, somewhat surprising that we found clear COHb boosts after 75 mL inhalations. One possible explanation is that, in our study, subjects employed a two second breath-hold whereas this was not the case in Guyatt's investigation. This breath-hold may have given the opportunity for CO to diffuse towards the lungs, to avoid immediate exhalation and to be carried into the lungs by the next inhalation.

Recently, Pankow (2001) has discussed the mechanisms by which moderately volatile compounds such as nicotine would be deposited in the respiratory tract during inhalation. He identified four different processes and considered the likely contribution of each of these to the total nicotine uptake. One process he considered is the deposition of nicotine that is initially in the gaseous phase. Pankow correctly states that the contribution of this mechanism must be small because there is very little nicotine in the gaseous phase when the aerosol leaves the cigarette (Houseman 1973, Stevens and Borgerding 1999). The second is the uptake of nicotine that evaporates from the aerosol particles during inhalation. The other two processes he discussed involve the deposition of nicotine from aerosol particles after they have deposited in the respiratory tract, one involving evaporation of nicotine from deposited particles and the other resulting from direct diffusion of nicotine from deposited particles into tissues. Pankow stated that the relative contributions of the various deposition mechanisms have not been determined for major brands when the smoke is inhaled according to typical smoker inhalation patterns. The results reported in our paper provide evidence that nicotine evaporation makes a major contribution to total nicotine deposition.

Conclusion

Our finding of that there is much greater retention of nicotine than solanesol with shallow inhalations is consistent with the view that nicotine evaporates from the cigarette smoke aerosol particles in the upper respiratory tract.

There is significant retention of nicotine in the mouth but this nicotine does not enter the systemic circulation.

Even a shallow inhalation (<250mL) results in 96% nicotine retention.

There is minimal capacity for smokers to increase nicotine retention by employing longer breath-hold durations or increased inhalation volumes.

In contrast, the particulate-based compound solanesol shows progressively increasing retention with breath-hold time and with inhalation volume.